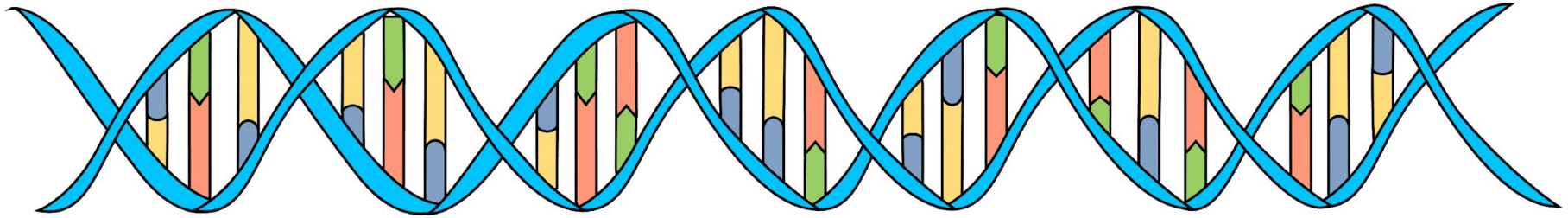
DNA Technology



Material Covered

The Polymerase Chain Reaction

1. DNA Replication in PCR
2. The PCR Process
3. Applications of PCR

DNA Profiling & Gel Electrophoresis

1. DNA Profiling/DNA Fingerprinting
2. DNA Electrophoresis

Material Covered

Genetically Modified Organisms

1. Process of Genetic Modification
2. Marker Genes

The Polymerase Chain Reaction

Specification Points

AQA

3.8.4.1 Recombinant DNA technology (A-level only)

Content	Opportunities for skills development
Recombinant DNA technology involves the transfer of fragments of DNA from one organism, or species, to another. Since the genetic code is universal, as are transcription and translation mechanisms, the transferred DNA can be translated within cells of the recipient (transgenic) organism. Fragments of DNA can be produced by several methods, including: • conversion of mRNA to complementary DNA (cDNA), using reverse transcriptase • using restriction enzymes to cut a fragment containing the desired gene from DNA • creating the gene in a 'gene machine'. Fragments of DNA can be amplified by *in vitro* and *in vivo* techniques. The principles of the polymerase chain reaction (PCR) as an *in vitro* method to amplify DNA fragments. The culture of transformed host cells as an *in vivo* method to amplify DNA fragments. • The addition of promoter and terminator regions to the fragments of DNA. • The use of restriction endonucleases and ligases to insert fragments of DNA into vectors. Transformation of host cells using these vectors. • The use of marker genes to detect genetically modified (GM) cells or organisms. (Students will **not** be required to recall specific marker genes in a written paper.)	**AT g** Students could investigate the specificity of restriction enzymes using extracted DNA and electrophoresis.

OCR

6.1.3 Manipulating genomes

	Learning outcomes	Additional guidance
(d)	the principles of the polymerase chain reaction (PCR) and its application in DNA analysis	

Specification Points

Edexcel A

Topic 6: Immunity, Infection and Forensics

Students should:
6.4 Know how DNA can be amplified using the polymerase chain reaction (PCR).

Edexcel B

Topic 7: Modern Genetics

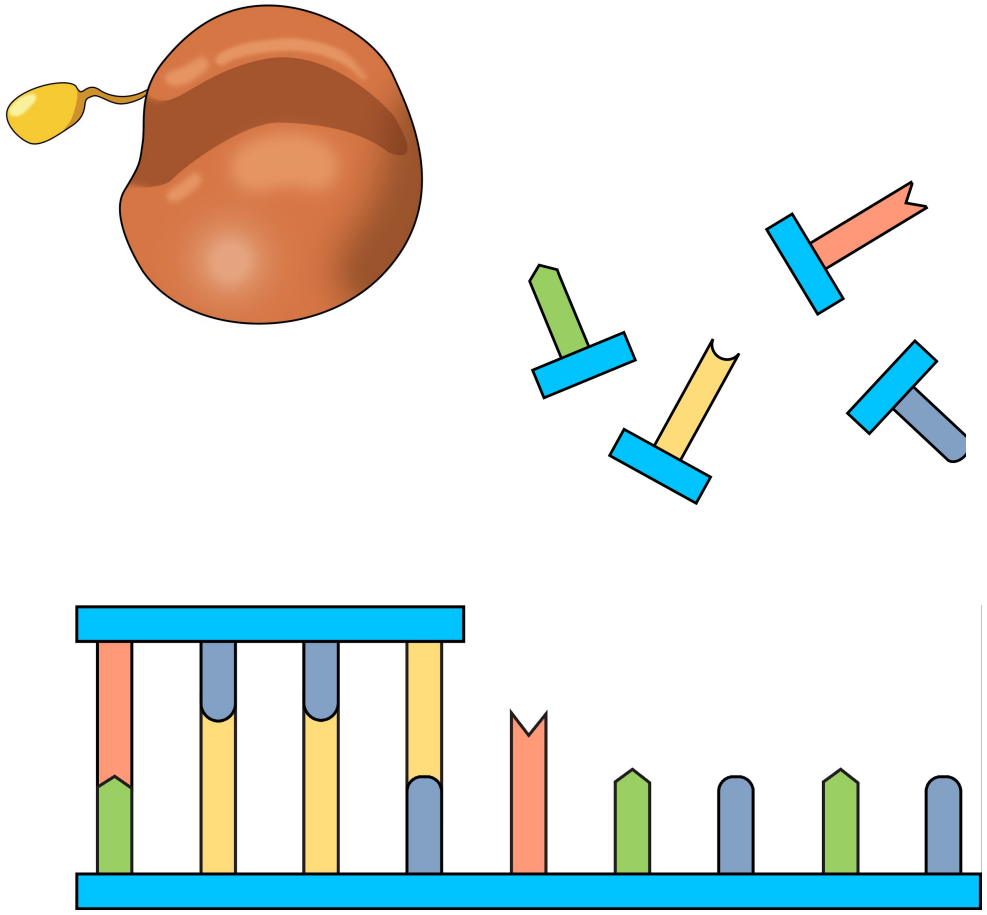
Students should:	
7.1	**Using gene sequencing**
ii	Understand how PCR can be used to amplify DNA samples, and how these samples can be used: • to predict the amino acid sequence of proteins and possible links to genetically determined conditions, using gene sequencing. • in forensic science, to identify criminals and to test paternity, using DNA profiling.

PCR Reactants

- PCR is used to **amplify DNA** *in vitro*, allowing production of lots of **DNA** from a **small sample**

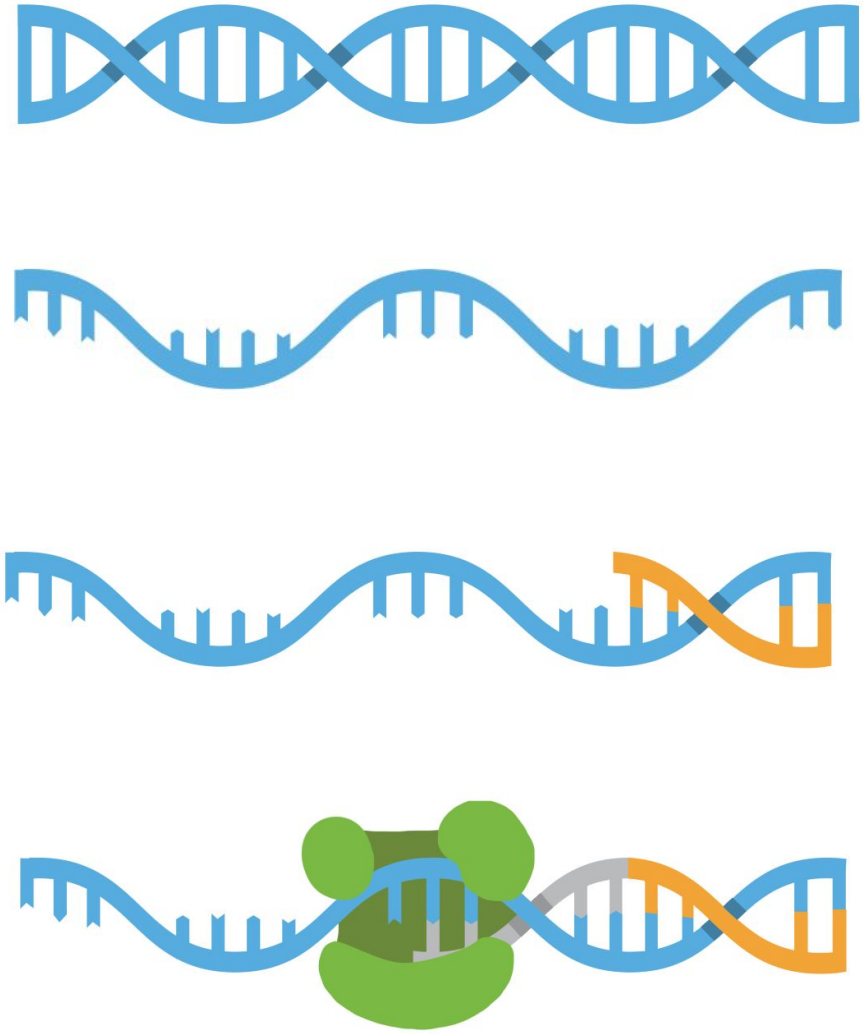
- PCR requires:

  - A **DNA sample** (max 1000bp)
  - **DNA Nucleotides** (A, T, G and C)
  - **DNA Polymerase** (Taq polymerase)
  - **Primers** (complementary to 3’ end of DNA sample)
  - A **thermocycler**



Stages of PCR

- PCR has **three** main stages:
  - **Denaturation**
    - **Separation** of the sample strands
  - **Annealing**
    - Binding of **primers** to separated strands
  - **DNA synthesis**
    - **Polymerase** synthesises new strand



Exemplar Exam Question – Statement

1) What are the three main stages in PCR?

[1 mark]

Command: simple recall

Direction: names of stages of PCR

Context: PCR reaction

Exemplar Exam Question – Statement

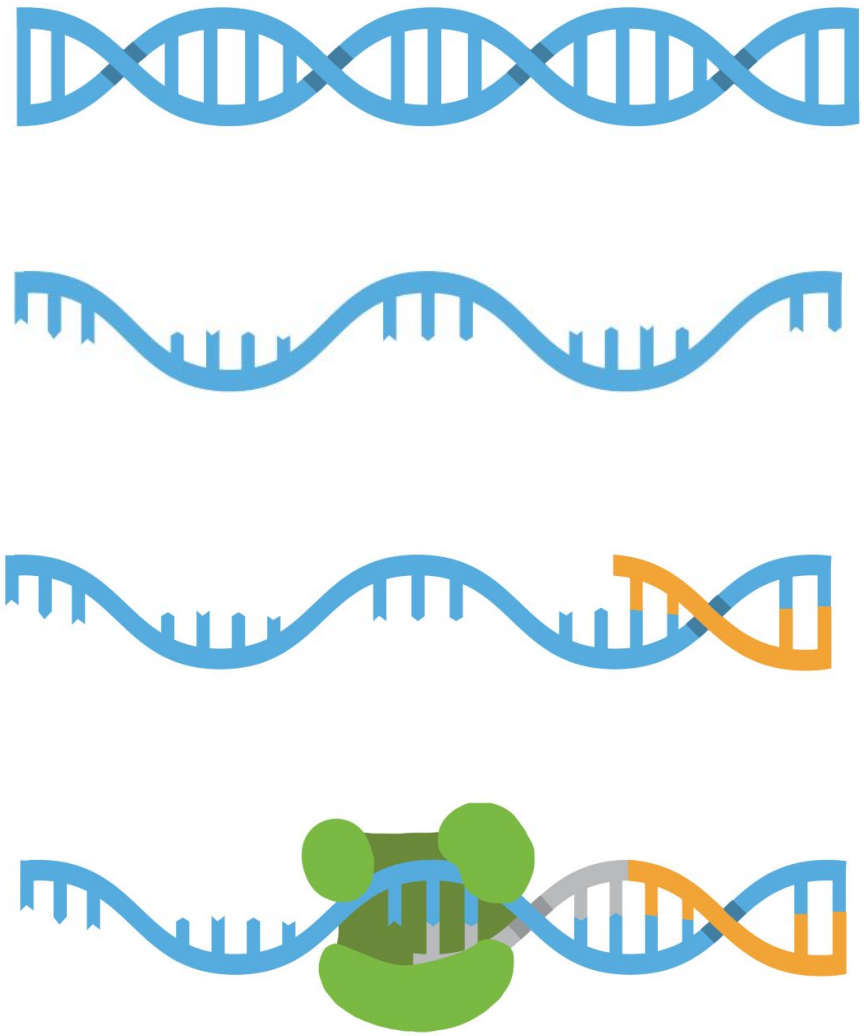
1) What are the three main stages in PCR?

[1 mark]

Denaturation, annealing and DNA synthesis.

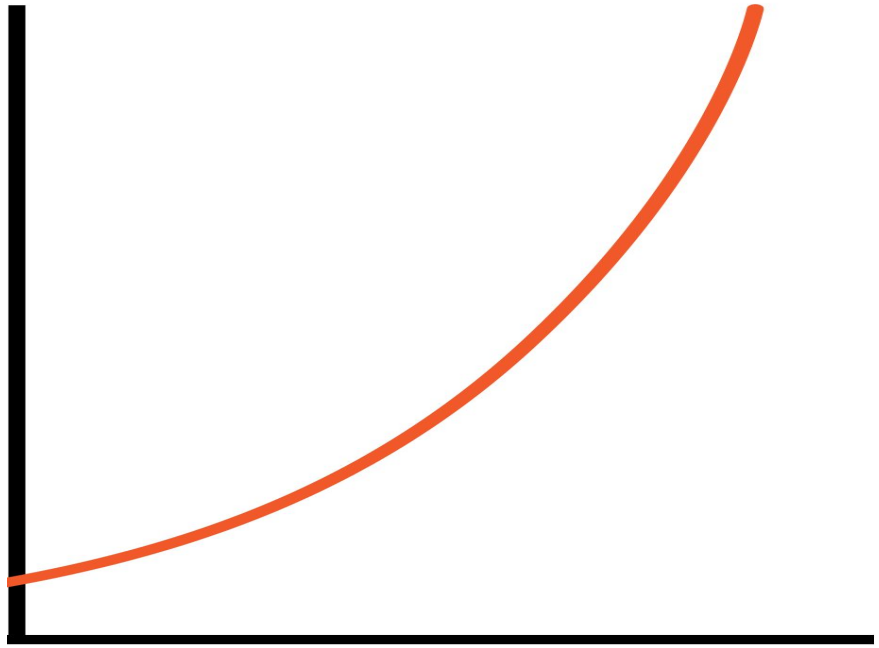
The Mechanisms of PCR

- The PCR reactants are **mixed**, placed in a **thermocycler** and heated to **95°C**, separating the strands of the **DNA** sample
- The temperature is decreased to **55-68°C** to allow **primer binding**
- The temperature is then raised to **70-72°C** allowing **DNA synthesis** to occur
- This results in **duplication** of the **sample DNA** – this is subsequently **repeated** many times to produce many new DNA strands



Exponential Amplification of DNA

- After each **cycle** the number of double-stranded **DNA molecules doubles**
- The number of DNA molecules therefore increases **exponentially**
- **$n = 2^x$** where **x** is the number of cycles, and **n** is the number of DNA molecules
- Each cycle takes **~2 minutes**, so billions of **DNA molecules** can be produced in a few hours



Exemplar Exam Question – Calculation

2) A PCR reaction is used to amplify a sample of DNA. The reaction begins with 16 molecules of DNA, and 23 cycles are completed.

Calculate the final number of molecules of DNA produced after the PCR has finished. Give your answer to the nearest million.

[2 marks]

Command: show your working

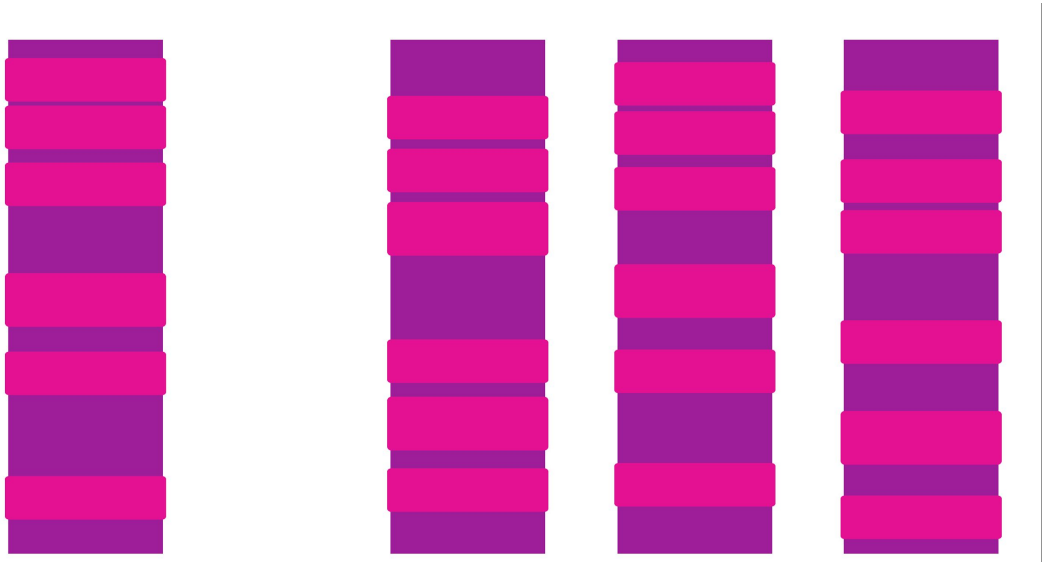
Direction: use the formula $n = 2^x$ and round final answer

Context: PCR exponential calculations

Exemplar Exam Question – Calculation

Applications of PCR

- **DNA** must be **amplified** before it can be used for:
  - **DNA sequencing** – determining cancer-causing **mutations** in patients
  - **DNA profiling/fingerprinting** – **matching DNA** from crime scenes with potential suspects
  - **Genetic engineering** – generating **genetically modified organisms**



Exemplar Exam Question – Simple Explanatory

3) Explain the importance of primers in the polymerase chain reaction (PCR).

[2 marks]

Command: short explanation, can bullet point

Direction: importance of primers in PCR

Context: PCR

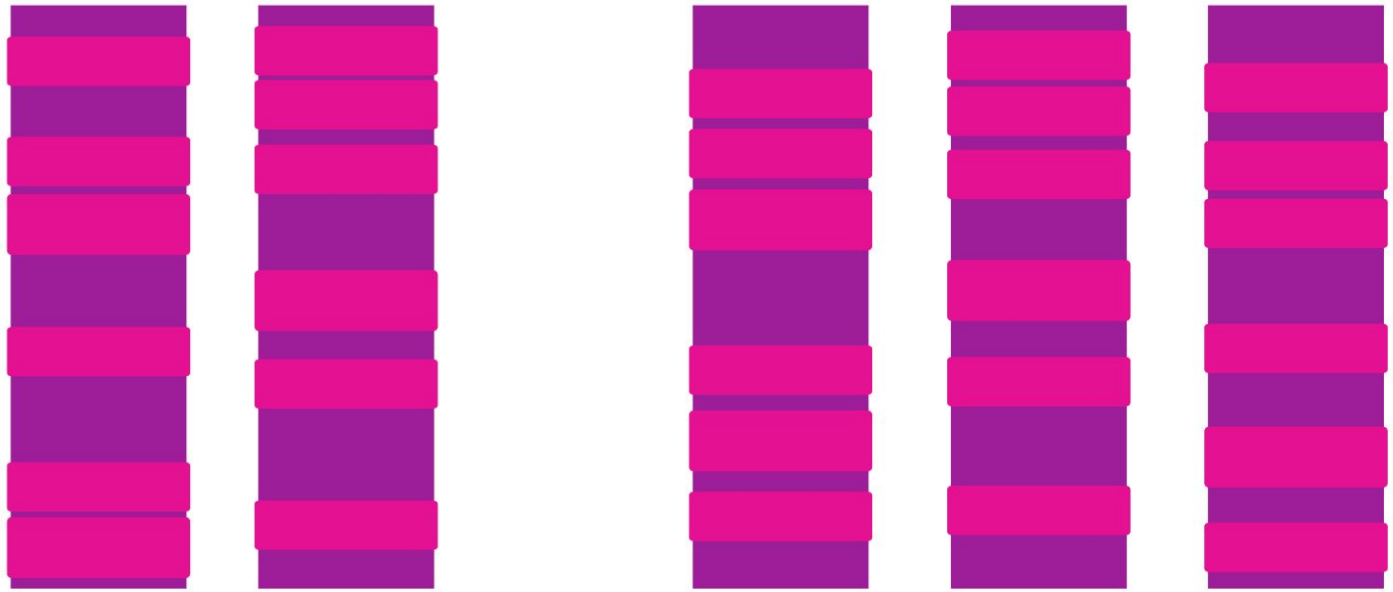
Exemplar Exam Question – Simple Explanatory

3) Explain the importance of primers in the polymerase chain reaction (PCR).

[2 marks]

Primers are complementary to the 3' end of each separated strand of DNA, allowing them to anneal. This creates a double-stranded area which Taq/DNA polymerase can bind to, allowing DNA synthesis/elongation to occur. They also prevent DNA strands rebinding.

DNA Profiling & Gel Electrophoresis



Specification Points

AQA

3.8.4.3 Genetic fingerprinting (A-level only)

Content	Opportunities for skills development
An organism's genome contains many variable number tandem repeats (VNTRs). The probability of two individuals having the same VNTRs is very low. The technique of genetic fingerprinting in analysing DNA fragments that have been cloned by PCR, and its use in determining genetic relationships and in determining the genetic variability within a population. The use of genetic fingerprinting in the fields of forensic science, medical diagnosis, animal and plant breeding. **Students should be able to:** • explain the biological principles that underpin genetic fingerprinting techniques • interpret data showing the results of gel electrophoresis to separate DNA fragments • explain why scientists might use genetic fingerprinting in the fields of forensic science, medical diagnosis, animal and plant breeding.	**AT g** Students could use gel electrophoresis to produce 'fingerprints' of food dyes.

AQA: Use the term VNTR

OCR

6.1.3 Manipulating genomes

	Learning outcomes	Additional guidance
(c)	the principles of DNA profiling and its uses	To include forensics and analysis of disease risk. HSW9
(e)	the principles and uses of electrophoresis for separating nucleic acid fragments or proteins	Opportunity for practical use of electrophoresis. **PAG6** HSW4

OCR: Use the term STR

Specification Points

Edexcel A

Topic 6: Immunity, Infection and Forensics

Students should:	
6.3	Know how DNA profiling is used for identification and determining genetic relationships between organisms (plants and animals).

Edexcel A: Use the term STR

Edexcel B

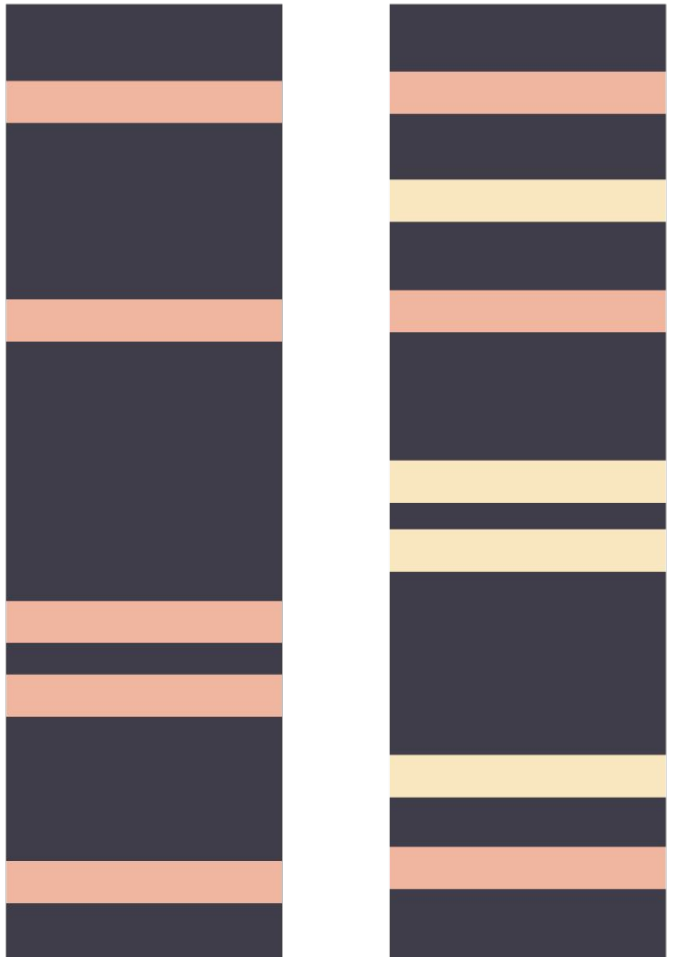
Topic 7: Modern Genetics

Students should:	
7.1	**Using gene sequencing**
ii	Understand how PCR can be used to amplify DNA samples, and how these samples can be used: • to predict the amino acid sequence of proteins and possible links to genetically determined conditions, using gene sequencing. • in forensic science, to identify criminals and to test paternity, using DNA profiling.

Edexcel B: Use the term microsatellites and minisatellites

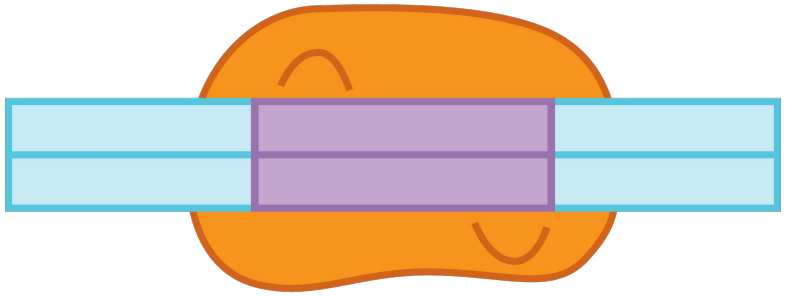
DNA Profiling/Genetic Fingerprints

- DNA profiling involves producing a specific **banding pattern** of **DNA** which is unique to **each individual**
- It uses **short repeating** sequences of DNA called **variable number tandem repeats** (**VNTRs**) or **short tandem repeats** (**STRs**) to produce this **banding pattern**
- Every individual **differs** in the **number** of **repeats** and therefore **length** of **VNTRs/STRs** so **DNA profiles** can be used to determine relatedness

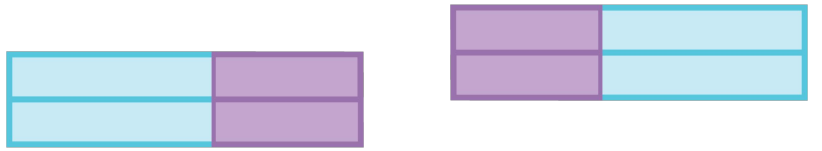


The Process of DNA Profiling

- DNA is cut into **fragments** using **restriction endonucleases/enzymes** that leave the **VNTRs/STRs** intact – forming **differently sized fragments**



- Restriction enzymes/endonucleases cut up DNA at a **specific sequence** of **bases** known as a **recognition site**



- When these fragments are **separated** out by **gel electrophoresis**, this produces a **unique banding pattern** which is visualised with **dyes** or **probes**

Exemplar Exam Question – Statement

4) What is the name of the small fragments which produce the banding pattern used in DNA profiling?

[1 mark]

Command: simple recall

Direction: give name only

Context: DNA profiling

_____

_____

Exemplar Exam Question – Statement

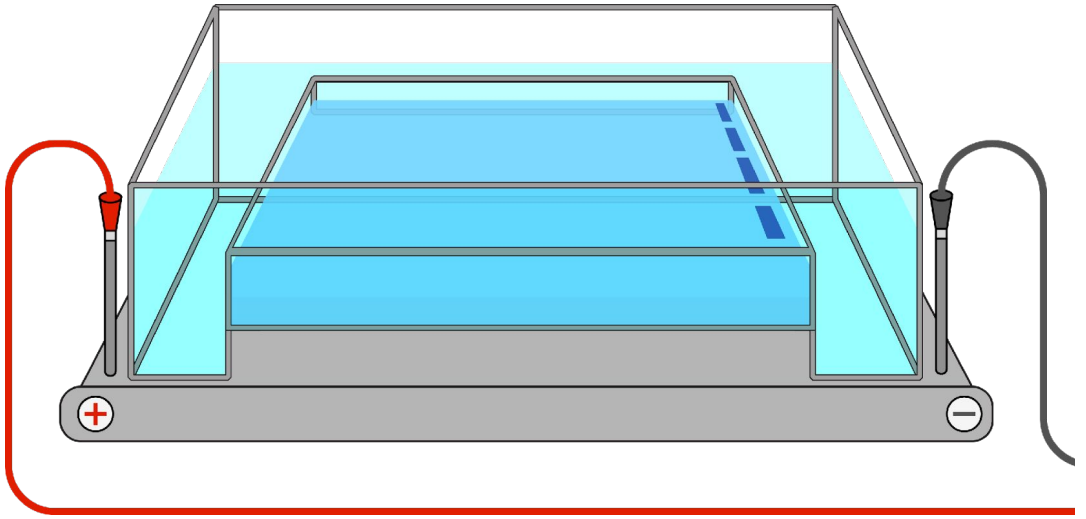
4) What is the name of the small fragments which produce the banding pattern used in DNA profiling?

[1 mark]

STRs/VNTRs/minisatellites/microsatellites

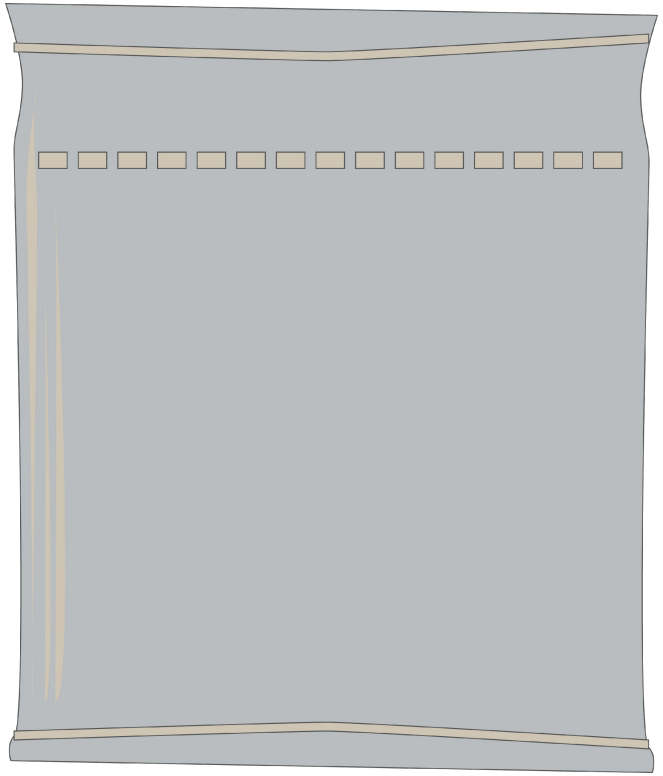
Separating DNA

- Electrophoresis separates out **macromolecules** by their **mass and charge**, using an **electric current**
- **DNA gel electrophoresis** separates **DNA** by **mass**, utilizing the **negatively charged phosphate backbone**
- DNA gel electrophoresis requires: a **digested DNA sample**, an **agarose gel plate**, **loading dye**, a **DNA ladder** and an **electrophoresis tank**



Process of DNA Gel Electrophoresis

- **DNA loading dye** is added to PCR tubes containing DNA samples, and they are pipetted into **wells** in the **agarose plate**, along with a **DNA ladder**
- An **electric current** is applied across the gel causing the DNA to move to the positive **anode**
- Smaller fragments **move faster**, and **travel further** in a given time period
- Addition of **DNA binding dye** after the gel has been run allows **visualization** of the **DNA**



Exemplar Exam Question – Explanatory

5) DNA profiling can be used to compare the genomes of different people. It is used in forensics and for paternity tests.

Explain the role of gel electrophoresis in DNA profiling.

[3 marks]

Command: more detailed response, critical thought required

Direction: define DNA profiling, explain gel electrophoresis and link

Context: DNA profiling, gel electrophoresis

Exemplar Exam Question – Explanatory

5) DNA profiling can be used to compare the genomes of different people. It is used in forensics and for paternity tests.

Explain the role of gel electrophoresis in DNA profiling.

[3 marks]

Gel electrophoresis is used to separate fragments of DNA by their size, generating a readable fingerprint that is unique to every individual. Different DNA samples can be compared to find commonalities in the gel bands.

Genetically Modified Organisms

Specification Points

AQA

3.8.4.1 Recombinant DNA technology (A-level only)

Content	Opportunities for skills development
Recombinant DNA technology involves the transfer of fragments of DNA from one organism, or species, to another. Since the genetic code is universal, as are transcription and translation mechanisms, the transferred DNA can be translated within cells of the recipient (transgenic) organism. Fragments of DNA can be produced by several methods, including: • conversion of mRNA to complementary DNA (cDNA), using reverse transcriptase • using restriction enzymes to cut a fragment containing the desired gene from DNA • creating the gene in a 'gene machine'. Fragments of DNA can be amplified by *in vitro* and *in vivo* techniques. The principles of the polymerase chain reaction (PCR) as an *in vitro* method to amplify DNA fragments. The culture of transformed host cells as an *in vivo* method to amplify DNA fragments. • The addition of promoter and terminator regions to the fragments of DNA. • The use of restriction endonucleases and ligases to insert fragments of DNA into vectors. Transformation of host cells using these vectors. • The use of marker genes to detect genetically modified (GM) cells or organisms. (Students will **not** be required to recall specific marker genes in a written paper.)	**AT g** Students could investigate the specificity of restriction enzymes using extracted DNA and electrophoresis.

OCR

6.1.3 Manipulating genomes

		Learning outcomes	Additional guidance
(f)	(i)	the principles of genetic engineering	To include the isolation of genes from one organism and the placing of these genes into another organism using suitable vectors.
	(ii)	the techniques used in genetic engineering	To include the use of restriction enzymes, plasmids and DNA ligase to form recombinant DNA with the desired gene and electroporation. HSW2

Specification Points

Edexcel A

Topic 8: Grey Matter

Students should:
8.17 Know how drugs can be produced using genetically modified organisms (plants, animals and microorganisms).

Edexcel B

Topic 7: Modern Genetics

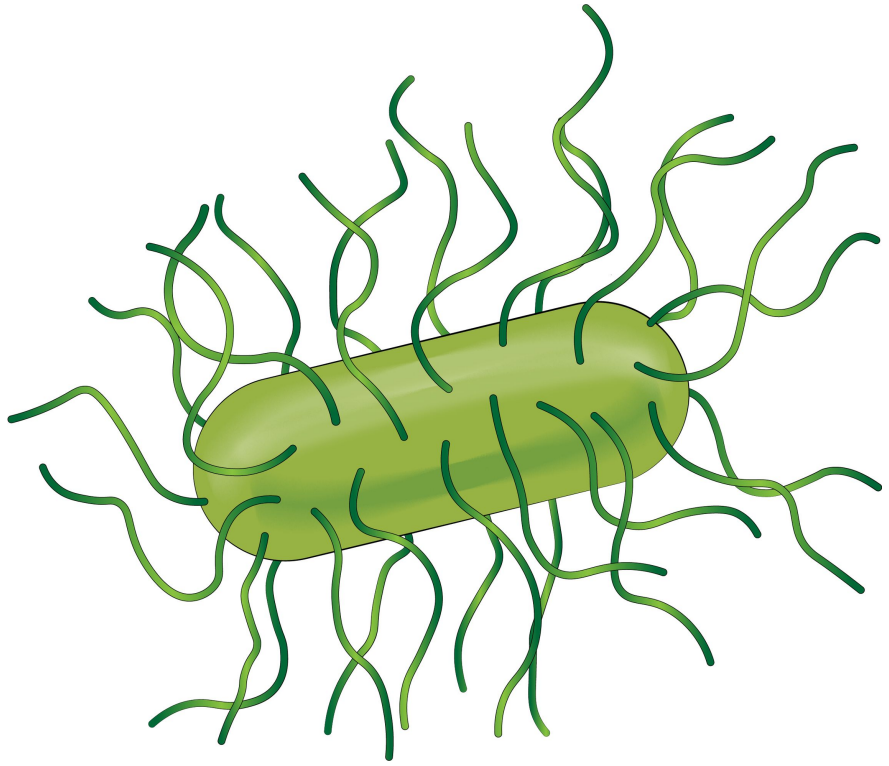
Students should:	
7.4	**Gene technology**
ii	Understand how recombinant DNA can be inserted into other cells, and the use of various vectors such as viruses and gene guns.

Uses of Genetically Modified Organisms

- **Transgenic organisms** are **GMOs** with **recombinant DNA**

Transgenic organisms are used to:

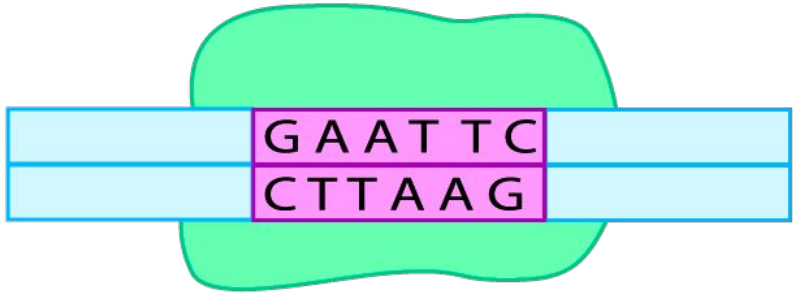
- Produce **antibiotics**, **hormones** and **enzymes** to treat **diseases**
- Produce **microorganisms** for **controlling pollution** or **increasing industrial efficiency**
- Increase **crop hardiness** and **yields**
- Provide specific **vitamins** and **chemicals** to prevent specific **diseases**

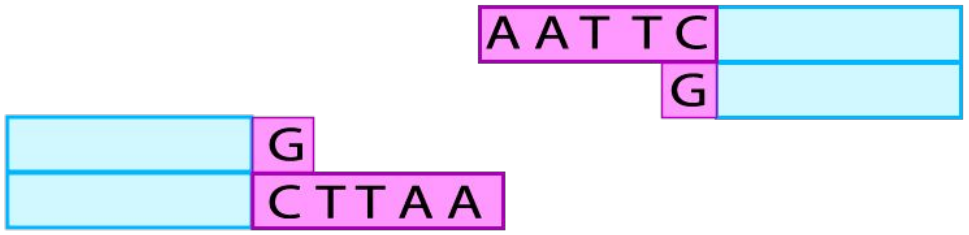


Isolating Genes

AQA: Need to know about promoters and terminators

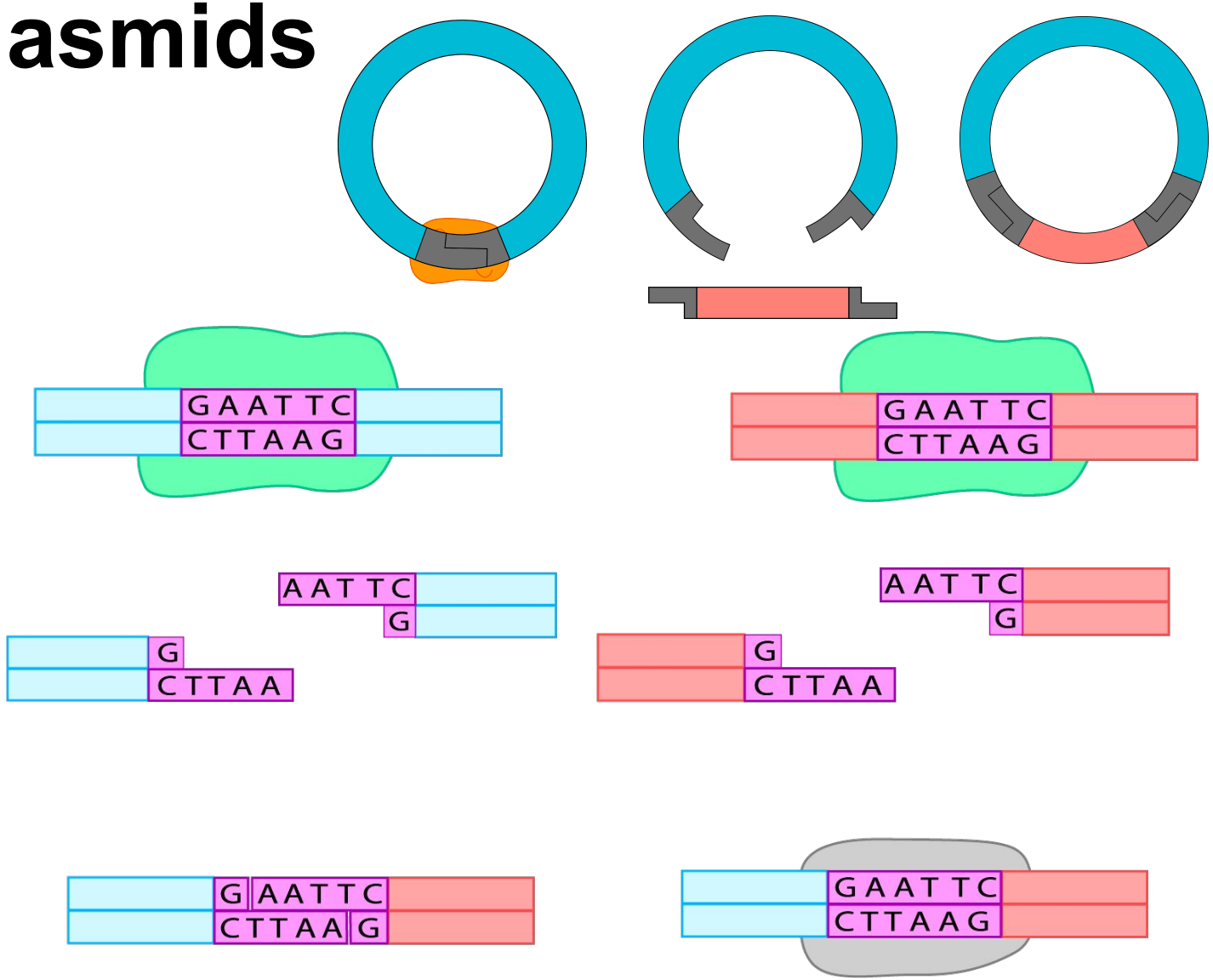
- **Desired genes** are **isolated** by being **cut** with **restriction enzymes**
- The **restriction enzymes** used create a **staggered cut**, resulting in **sticky ends**
- After **isolation**, **promotor regions** and **terminator regions** are also **added**
- **Other methods** of **isolation** are also possible such as by using **reverse transcriptase** or a **polynucleotide synthesiser**





Inserting Genes in Plasmids

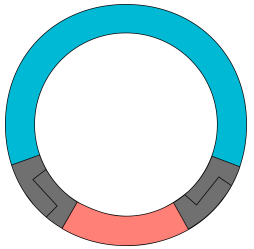
- **Plasmids** are used as **vectors** for **transferring genes** into **bacteria**
- Plasmid is **cut** open using the **same restriction enzyme** so **sticky** ends of **plasmid** and **gene** are **complementary**
- Once **annealed**, **DNA ligase** permanently incorporates the **gene** into the **plasmid**

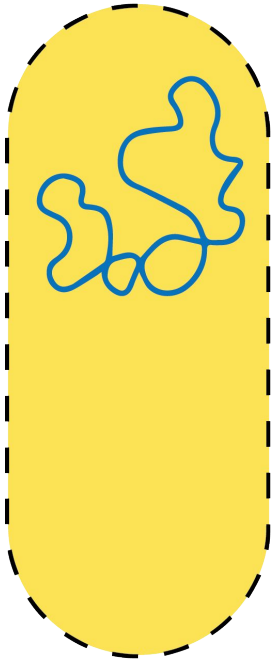


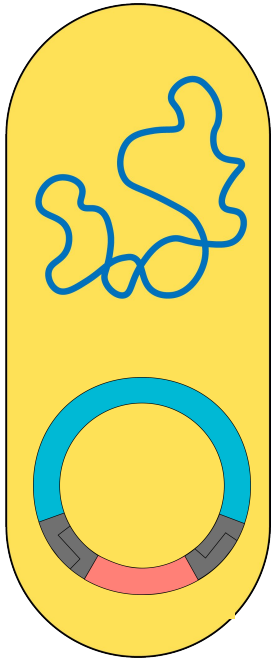
Transformation

OCR: Need to know about **electroporation**
AQA & OCR : Need to **know about Ca^{2+}**

- **Plasmids** are **inserted** into **bacteria cells** in a process known as **transformation**
- **Electric currents** or sudden **changes** in **temperature** create **pores** in the **bacterial cell membrane**
- The **plasmid** moves in through these **pores**
- The **solution** must contain **calcium ions** to **negate** the **repulsion** between **plasmid DNA** and the **cell membrane**







Exemplar Exam Question – Simple Explanatory

6) Explain why the same restriction enzymes must be used during genetic modification when cutting a gene and a plasmid it is to be ligated into.

[2 marks]

Command: short sentences, give reason

Direction: importance of how restriction enzymes cut DNA

Context: genetic modification, insertion into plasmid

Exemplar Exam Question – Simple Explanatory

6) Explain why the same restriction enzymes must be used when cutting a gene and a plasmid it is to be ligated into during genetic modification

[2 marks]

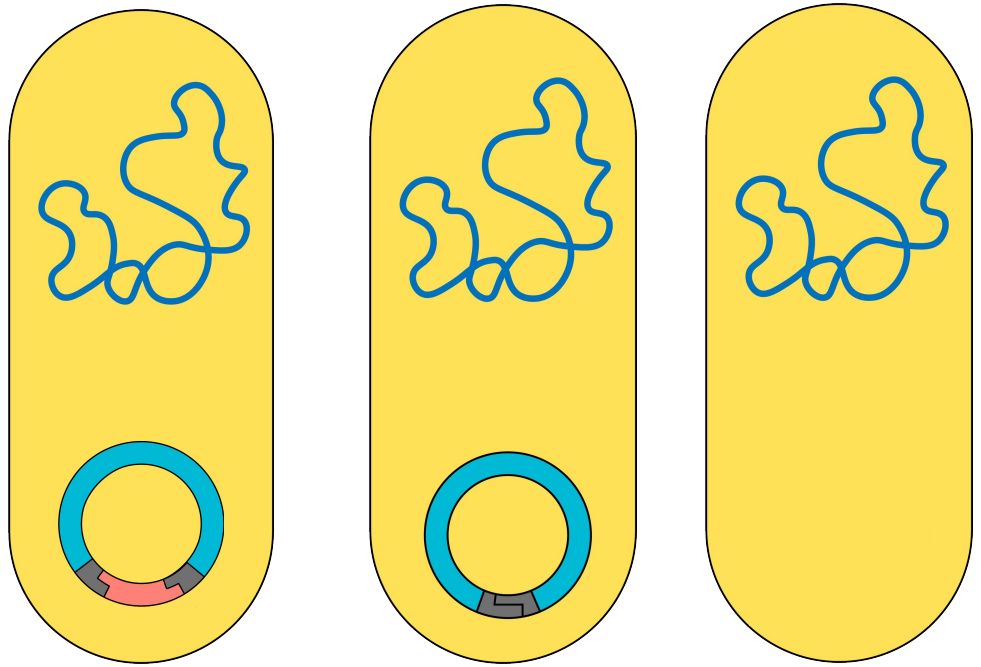
Using the same restriction enzymes to cut the gene and plasmid means that the same restriction sites are cut, producing identical sticky ends. The sticky ends are therefore complementary, allowing the DNA to anneal.

Marker Genes

- **Marker genes** are required as **transformation** is often **not successful** – **bacteria** may take up an **empty plasmid** or **no plasmid** at all

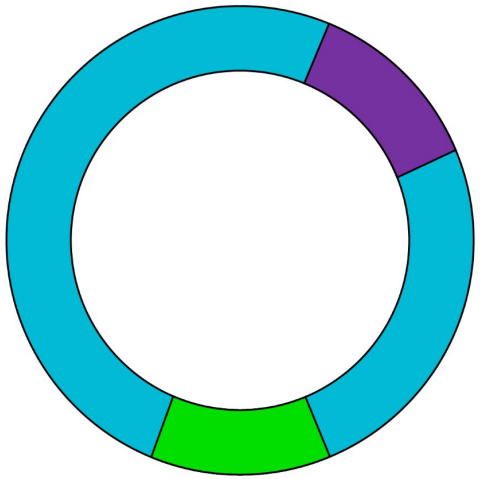
- The **plasmids** used as **vectors** contain marker genes which have **easily identifiable phenotypes**

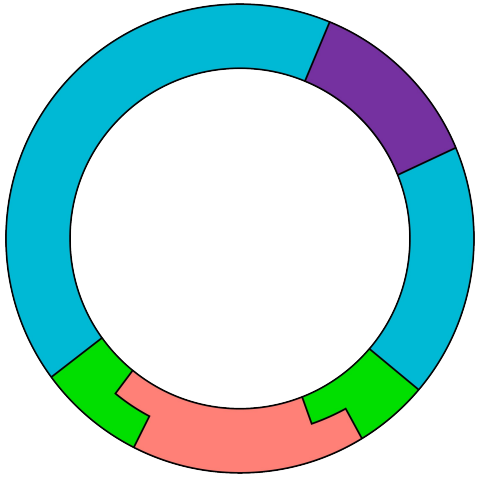
- Examples include genes for:
  - **Antibiotic resistance**
  - **Fluorescent proteins**
  - An **easily identifiable enzyme**



Identifying GM Bacteria

- **Plasmids** usually contain **two marker genes**
- The **first marker gene** is a **functional gene** found in the **plasmid** but the **second marker gene** is **non-functional** as the **desired gene** is **inserted** into it
- **GM bacteria** with **recombinant plasmid** therefore have the **phenotype** of the **first marker gene** but **not** the **phenotype** of the **second marker gene**

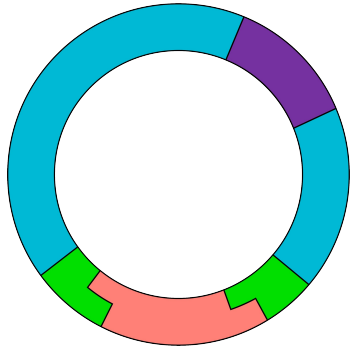


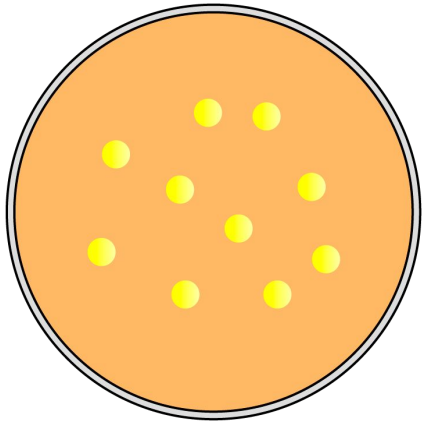


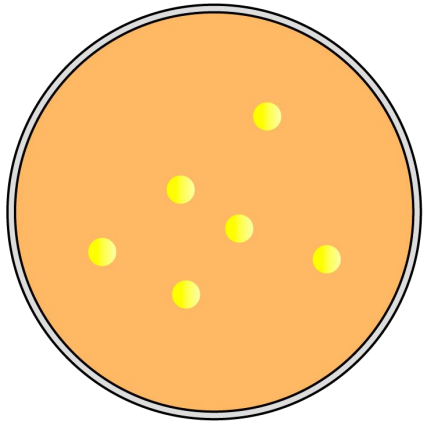
AQA & OCR: Need to know about the 2 marker system

Antibiotic-resistance Marker Genes

- The **phenotypes** of **antibiotic-resistant genes** can be seen by growing **bacteria** on **petri dish** that contains that **antibiotic**
- When the **antibiotic-resistance** is due to the **functional marker gene**, scientists **select** for it (select the **colonies** that do **not die**)
- The selected colonies are **replica plated** with the second antibiotic. Colonies that **die** are selected as their **second marker gene** is **non-functional** as the **desired gene** has been **successfully inserted** into it.

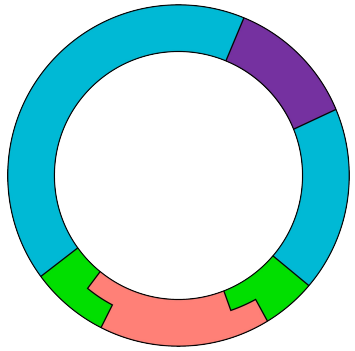


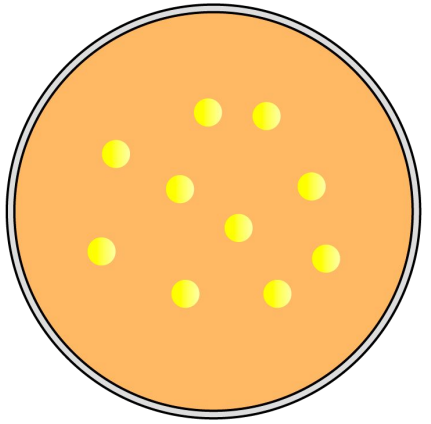


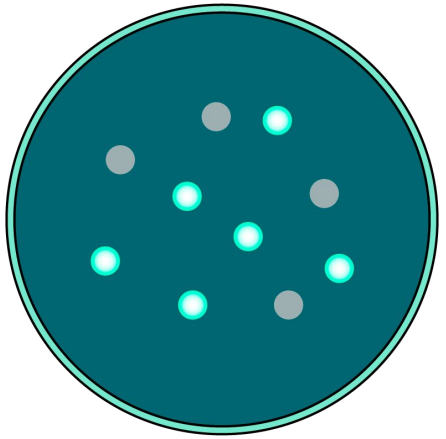


Fluorescent and Enzyme Marker Genes

- **Fluorescent protein marker genes** (e.g. **GFP**) cause cells to **fluoresce** under **UV light**
- **Enzymes** (e.g. **lactase)** can turn certain **substrates** (e.g. X-gal) blue resulting in **blue colonies**
- These are usually the **phenotypes** of the **non-functional marker gene**, and so scientists **select against** them







Exemplar Exam Question – Data Analysis

7) A scientist wanted to create genetically modified bacteria. He isolated his desired gene using the restriction enzyme BamH I. The scientist used the plasmid pBR322 as the vector for transforming the bacteria. The diagram for pBR322 is shown in Figure 1. After inserting the desired gene into pBR322, the scientist needed to identify which bacteria had successfully taken up the recombinant plasmid.

Using Figure 1 and your own knowledge, fill in the table to state what phenotypes the bacteria may show. Use a tick (✓) for functional phenotypes and a cross (X) for non-functional phenotypes.

[3 marks]

Exemplar Exam Question – Data Analysis

7) Using Figure 1 and your own knowledge, fill in the table to state what phenotypes the bacteria may show. Use a tick (✓) for functional phenotypes and a cross (X) for non-functional phenotypes.

[3 marks]

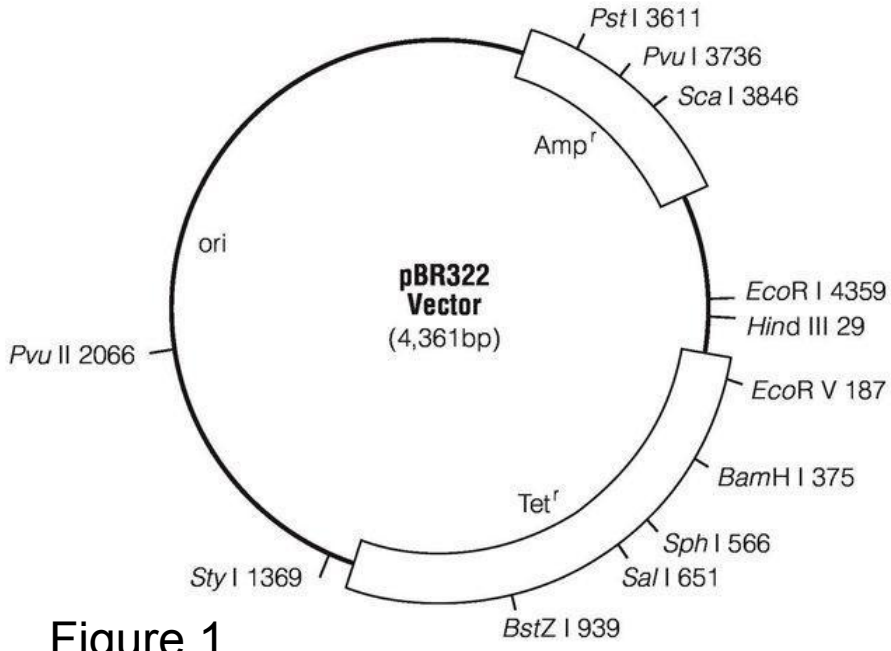


Figure 1

	Ampicillin Resistance	Tetracycline Resistance
Bacteria with no plasmid		
Bacteria with empty plasmid		
Bacteria with recombinant plasmid		

Exemplar Exam Question – Data Analysis

7) Using Figure 1 and your own knowledge, fill in the table to state what phenotypes the bacteria may show. Use a tick (✓) for functional phenotypes and a cross (X) for non-functional phenotypes.

[3 marks]

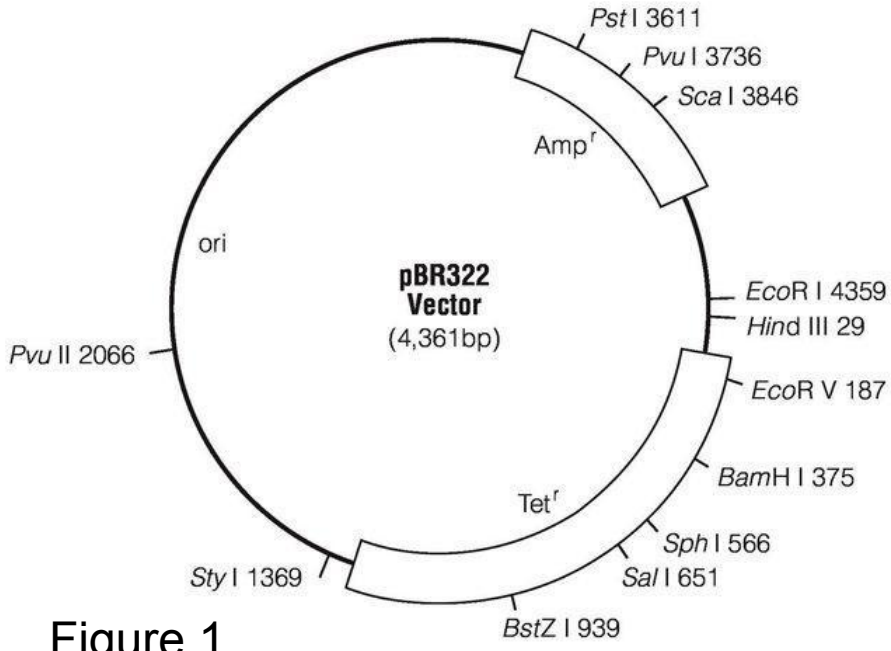


Figure 1

	Ampicillin Resistance	Tetracycline Resistance
Bacteria with no plasmid		
Bacteria with empty plasmid		
Bacteria with recombinant plasmid		

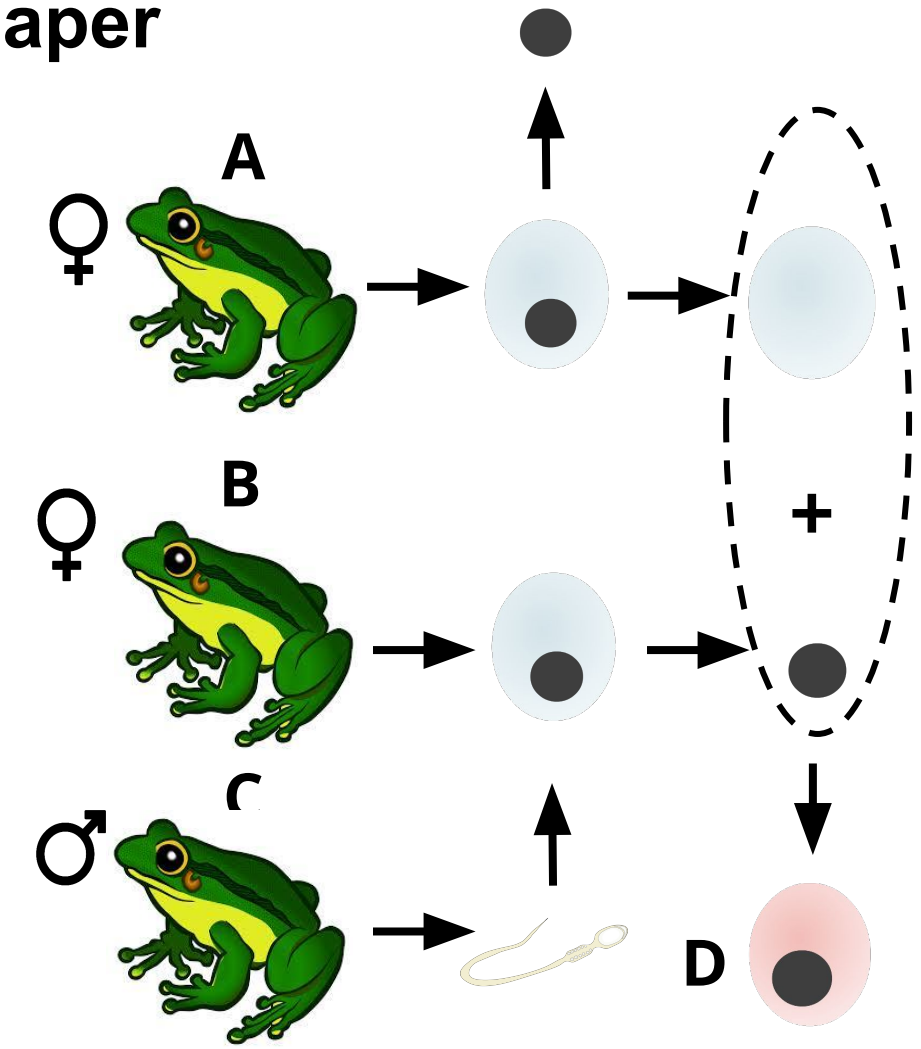
SNAPREVISE

Mini Mock Paper

Mini Mock Paper

a) Consider the following scenario:

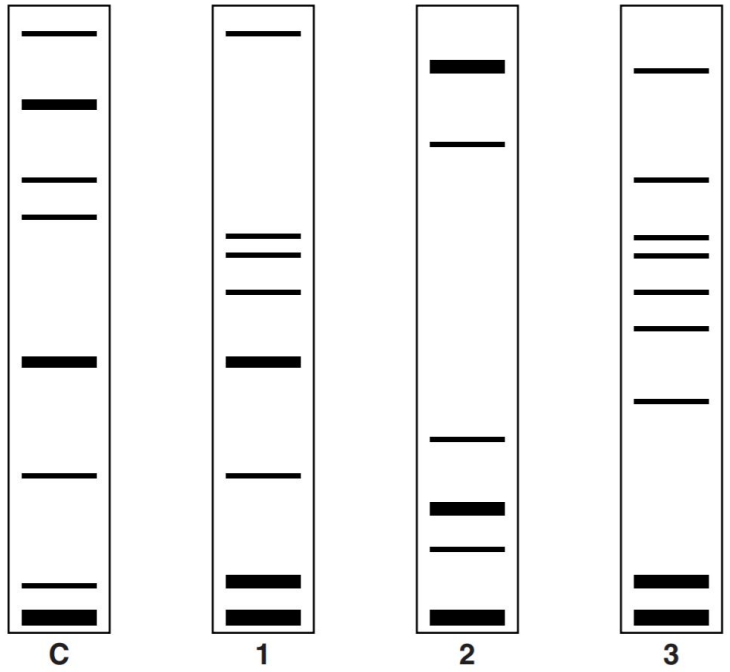
- **Female A** – Laid eggs, nuclei removed and discarded
- **Female B** – Laid eggs which were fertilised by Male C, nuclei placed into enucleated female A egg cells
- **Male C** – Sperm used to fertilize eggs from Female B
- **Offspring D** – Resultant offspring from **egg D**



a) Below are genetic profiles of frogs A, B, C and D (produced by gel electrophoresis)

Only C is labelled. Identify numbers 1, 2 and 3 as the remaining frogs.

[2 marks]



Number	Frog
1	
2	
3	

Mini Mock Paper

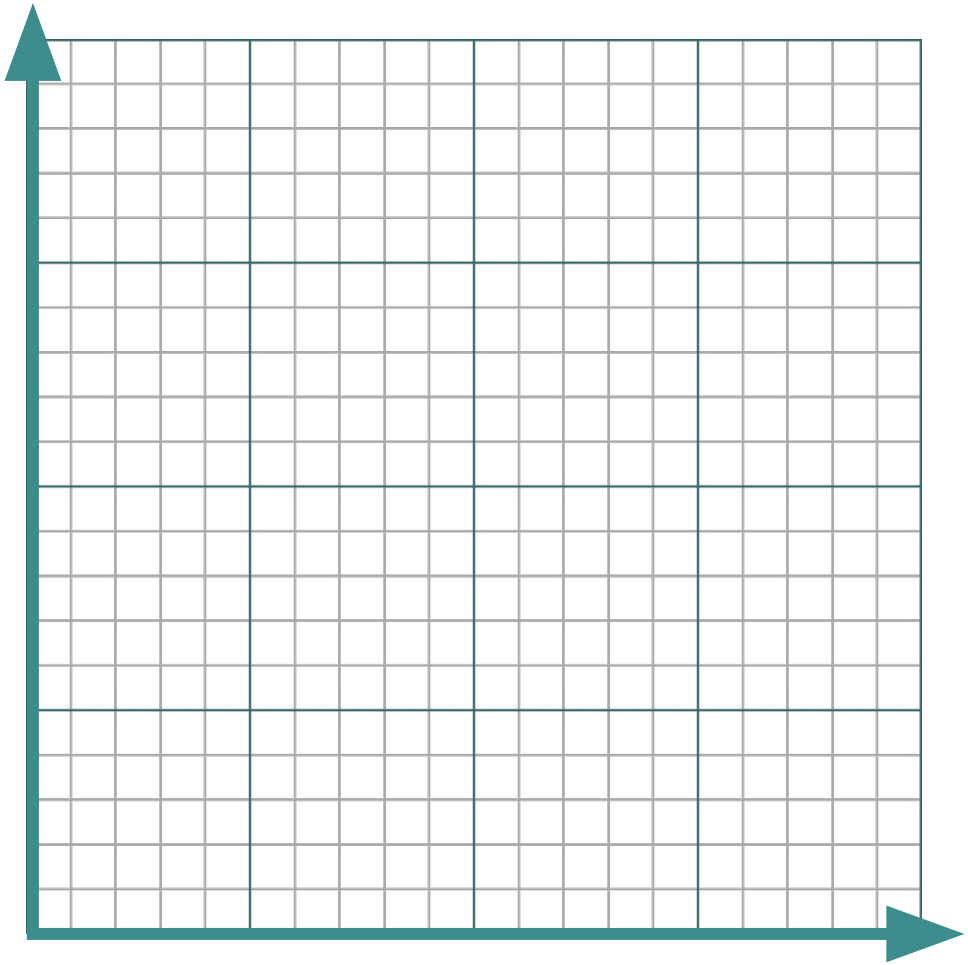
b) Standard DNA fragments of known length were separated by gel electrophoresis. The lengths of the fragments are measured in DNA base pairs. The table shows the distance migrated by the standard DNA fragments.

Fragment length (bp)	Distance migrated (mm)
10,000	6.1
8,000	9.5
6,000	15.5
4,000	21.8
2,000	36.0
1,000	50.0
500	61.2

Plot a graph of $\log_{10}$ fragment length against distance migrated.

[2 marks]

Mini Mock Paper



Mini Mock Paper

c) Use your graph to estimate the length of a fragment which migrates 44.0 mm in the same gel.

Fragment length (bp)	Distance migrated (mm)
10,000	6.1
8,000	9.5
6,000	15.5
4,000	21.8
2,000	36.0
1,000	50.0
500	61.2

[2 marks]

Mini Mock Paper

d) Evaluate the advantages and disadvantages of the use of DNA technology.

[6 marks]

Mini Mock Paper

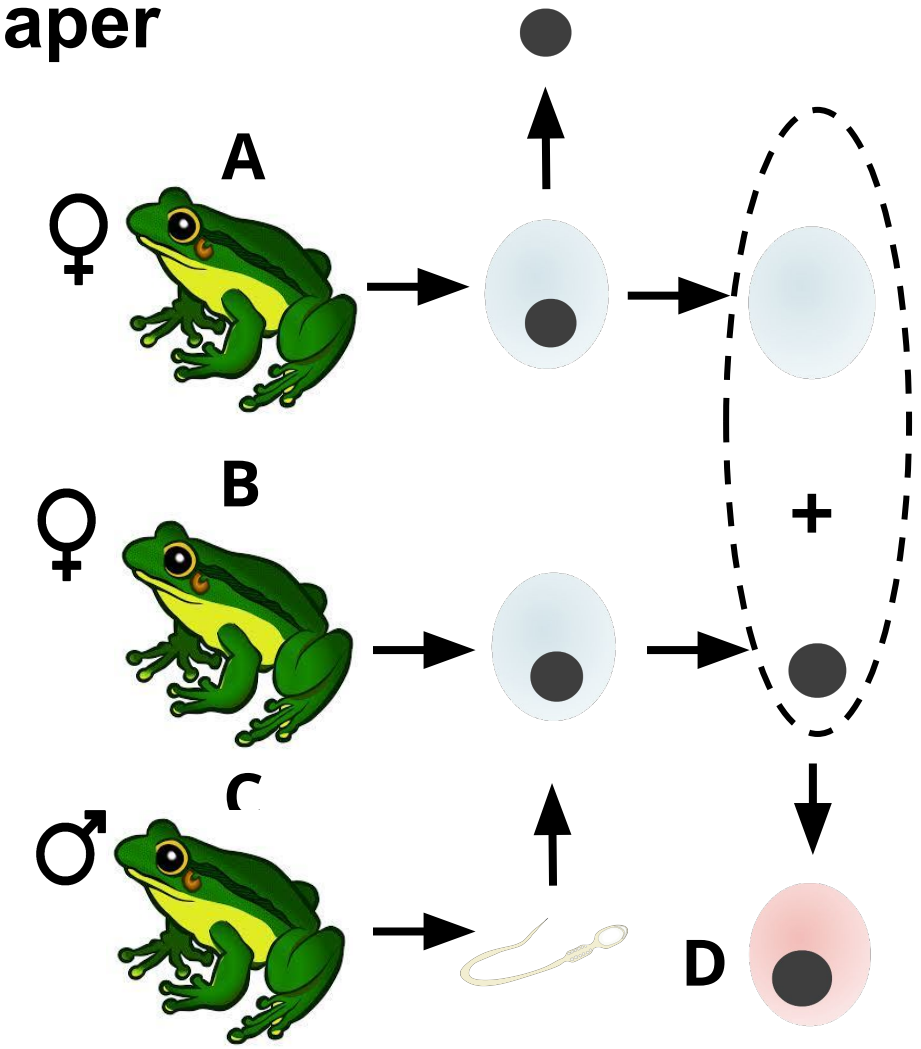
SNAPREVISE

Mini Mock Paper Answers

Mini Mock Paper

a) Consider the following scenario:

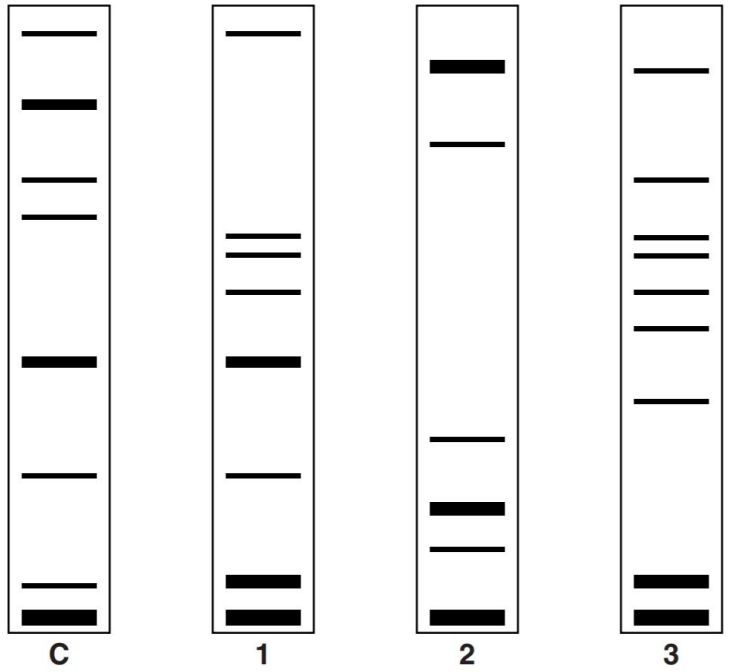
- **Female A** – Laid eggs, nuclei removed and discarded
- **Female B** – Laid eggs which were fertilised by Male C, nuclei placed into enucleated female A egg cells
- **Male C** – Sperm used to fertilize eggs from Female B
- **Offspring D** – Resultant offspring from **egg D**



a) Below are genetic profiles of frogs A, B, C and D (produced by gel electrophoresis)

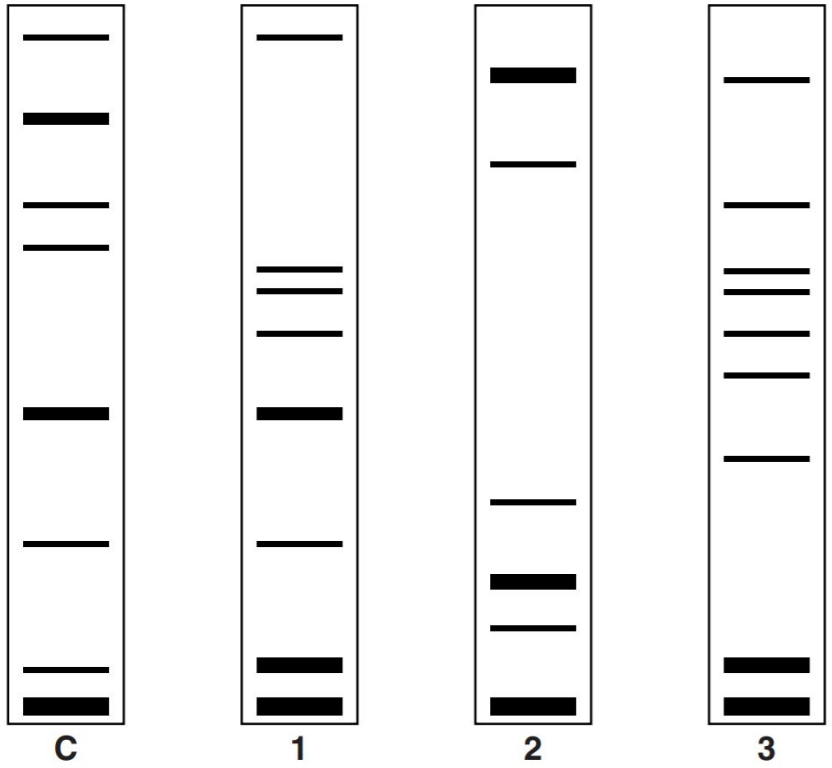
Only C is labelled. Identify numbers 1, 2 and 3 as the remaining frogs.

[2 marks]



Number	Frog
1	
2	
3	

Mini Mock Paper



Number	Frog
1	Offspring D
2	Female A
3	Female B

Mini Mock Paper

b) Standard DNA fragments of known length were separated by gel electrophoresis. The lengths of the fragments are measured in DNA base pairs. The table shows the distance migrated by the standard DNA fragments.

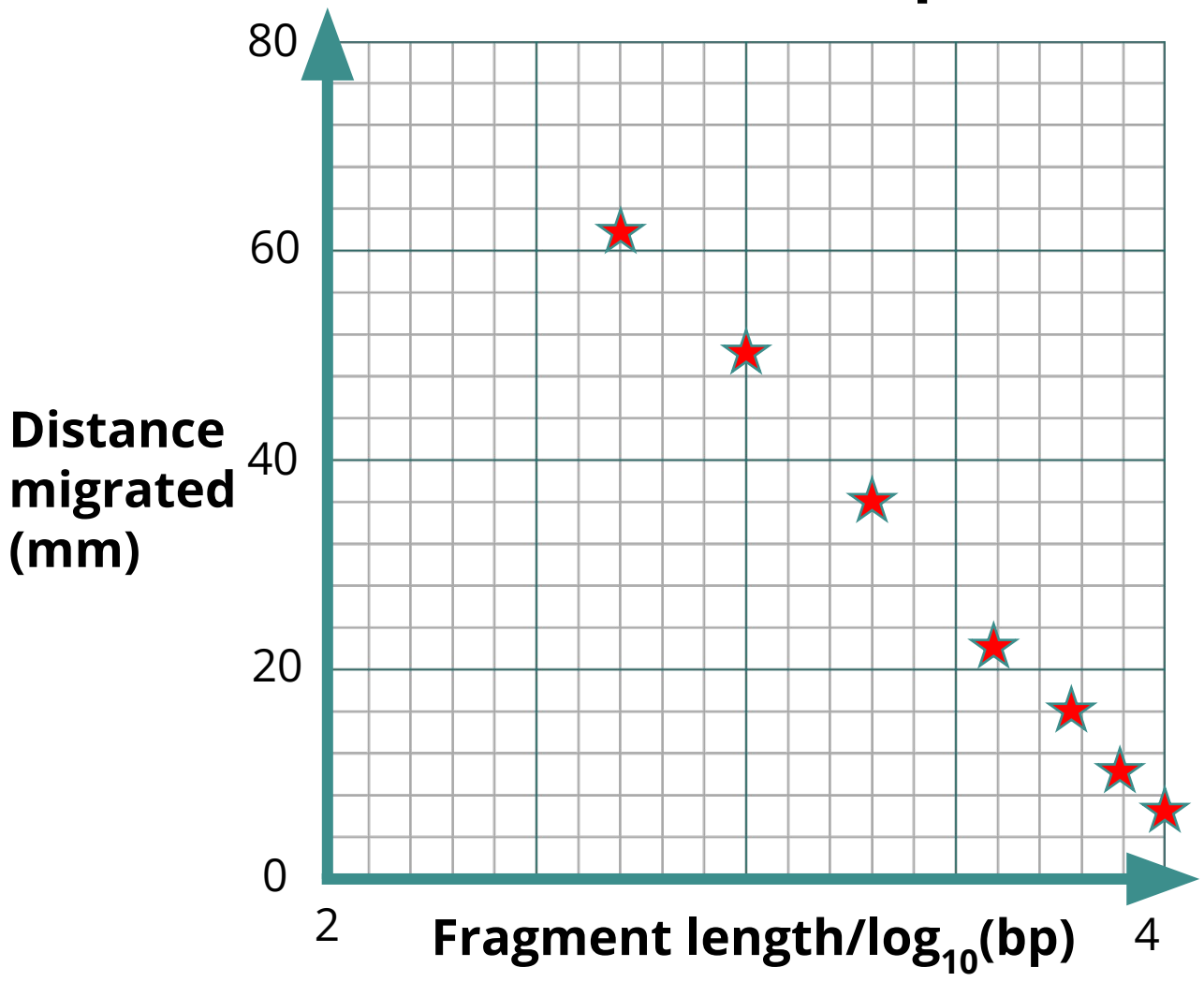
Fragment length (bp)	Distance migrated (mm)
10,000	6.1
8,000	9.5
6,000	15.5
4,000	21.8
2,000	36.0
1,000	50.0
500	61.2

Plot a graph of $\log_{10}$ fragment length against distance migrated.

.

[2 marks]

Mini Mock Paper



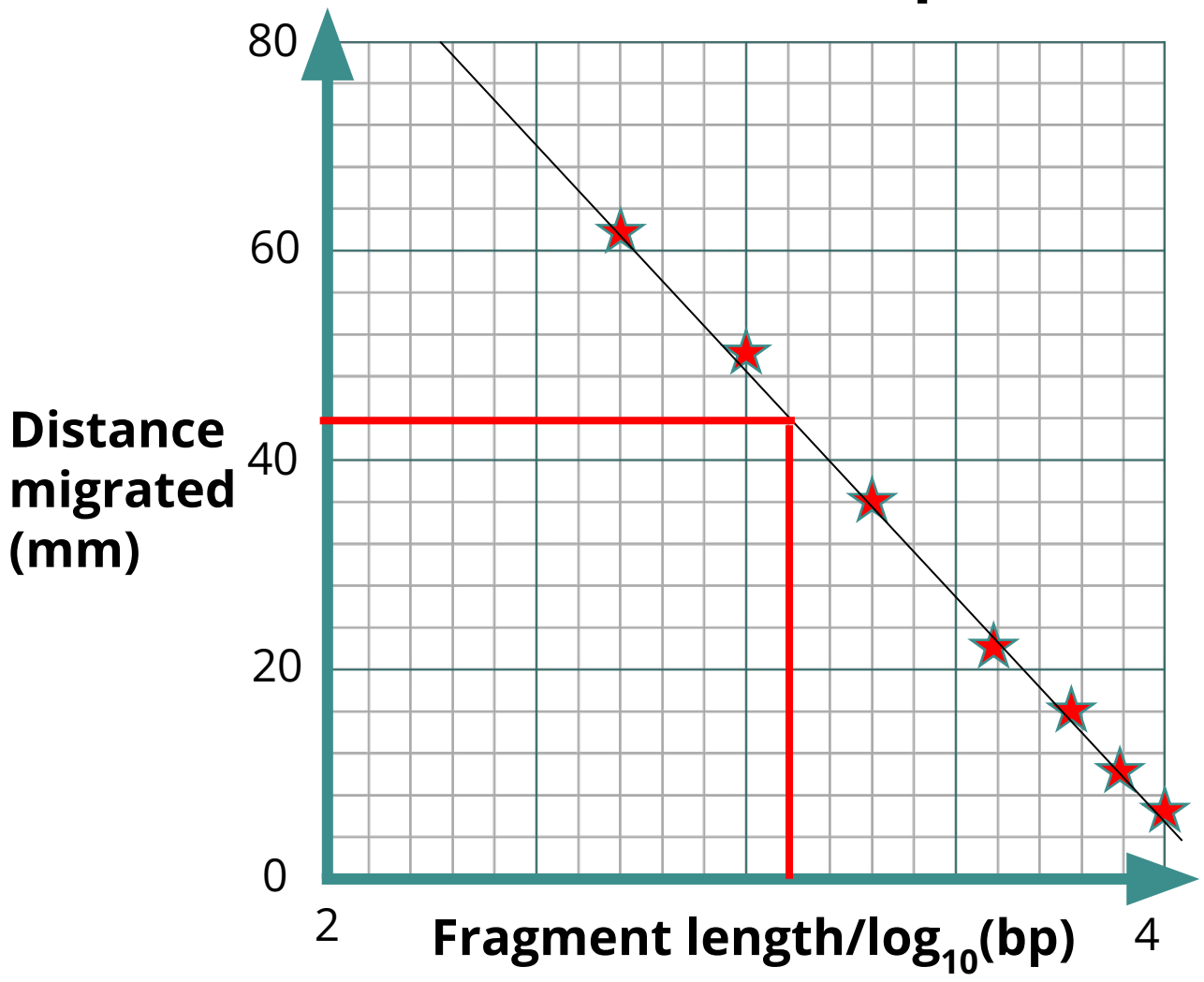
Mini Mock Paper

c) Use your graph to estimate the length of a fragment which migrates 44.0 mm in the same gel.

Fragment length (bp)	Distance migrated (mm)
10,000	6.1
8,000	9.5
6,000	15.5
4,000	21.8
2,000	36.0
1,000	50.0
500	61.2

[2 marks]

Mini Mock Paper



Mini Mock Paper

d) Evaluate the advantages and disadvantages of the use of DNA technology.

[6 marks]

DNA technology can be used to produce GM plants, animals and microorganisms which produce useful substances or have beneficial traits. GM microorganisms can be used to produce antibiotics, hormones and enzymes and GM crops can be produced which are drought or disease tolerant, producing higher crop yields whilst requiring fewer resources. GM crops can also be used to produce vitamins

Mini Mock Paper

which local populations lack – such as golden rice which contains high levels of vitamin A. Unfortunately the release of GMOs can have unintended consequences – release of antibiotic resistant bacteria into the environment can be risky. Genetic fingerprinting, another DNA technology, can be used to compare sample DNA against known sequences, allowing comparison of evidence at crime scenes with potential suspects, but it may be used to wrongly incriminate suspects by switching DNA samples. DNA technologies have the potential to be incredibly useful, but abuse and overuse may lead to issues, therefore it must be used with caution to prevent issues arising.